

Application No. 10/564,264
Attorney Docket No. 38523.000324

RECEIVED
CENTRAL FAX CENTER
JUL 25 2007

Rosetta(DE3)pLysS
Rosetta(DE3)pLysE
Rosetta(DE3)pLacI
Tuner(DE3)
Tuner(DE3)pLysS
Tune (DE3)pLacI
Group II: GroEL
GroEL/GroES
peptidyl-prolyl isomerase (PPI)
peptide disulfide isomerase (PDI)
Group IX: GroEL
GroES
SpeB pro-polypeptide domain
PDI
PPI

ELECTION

Applicants respectfully request withdrawal of the election requirement and ask that all groups and species be prosecuted in the same patent application. In the event that the election requirement is not withdrawn and solely in order to comply with 37 C.F.R. § 1.143, Applicants hereby provisionally elect the group as follows:

Group I, claims 1-22, methods for expressing mature SpeB, using a polycistronic plasmid which comprises a polynucleotide encoding SpeB polypeptide and mature SpeB polypeptide.

In addition, Applicants provisionally elect the species as follows:

Group I: BLR(DE3)

Applicants reserve the right to file divisional application(s) directed to non-elected subject matter and reserve the right to petition the election requirement. Upon allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species which

Application No. 10/564,264
Attorney Docket No. 38523.000324

depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR § 1.141.

RECEIVED
CENTRAL FAX CENTER
JUL 25 2007

TRAVERSAL

Applicants respectfully traverse the Examiner's restriction requirement, as follows. A requirement for restriction is only proper when a serious burden is placed on the Examiner. Applicants respectfully submit that a search and examination of all claims may be made without imposing a serious burden on the Examiner. Further, an important advantage in pursuing just one application encompassing all of the invention groups cited by the Examiner is that the examination work of the U.S. Patent and Trademark Office would be simplified, insofar as duplication of searching effort would be eliminated.

The Restriction Requirement violates the PCT rules and US restriction practice governing patent applications filed under 35 USC 371 since it is in conflict with Article 127(1) of the PCT wherein it is stated that no national law shall require compliance with requirements correlating to the form or context of the international application different from or additional to those in which are provided in the Treaty and the Regulations. Article 27(1) is further clarified in the PCT guidelines under Item 138, wherein it is stated that an international application which complies with the unity of invention requirement laid down in Rule 13 PCT must be accepted in all the designated and elected offices.

With respect thereto, Applicants note that this application is a US national filing under 35 USC 371 based on PCT application PCT/US2004/021714. Therefore, the Restriction Requirement herein must adhere to the governing PCT regulations. Applicants submit concurrently with this response copies of the International Preliminary Report on Patentability and the Written Opinion of the International Searching Authority. Both documents indicate that the International Searching Authority found no lack of unity of invention with the present claims. Contrary to the Examiner's alleged reasons for restriction, Applicants do not agree that the

Application No. 10/564,264
Attorney Docket No. 38523.000324

inventions listed in Groups I-X do not relate to a single inventive concept because they lack the same or corresponding technical features. The Examiner in fact states that the technical feature linking Groups I-X is a mature SpeB polypeptide. See Office Action, p. 3.

Therefore, Applicants respectfully request that Groups I-X should be rejoined and examined together in response to the Election response submitted herewith. All of the claims are believed to correspond to the elected subject matter which defines a unitary invention according to the PCT guidelines as described above.

Applicants respectfully reserve the right to traverse the Examiner's remarks regarding Eriksson et al., Infect. Immun., 71:211-217 (Jan 2003) if that reference is applied by the Examiner to reject any claims.

Application No. 10/564,264
Attorney Docket No. 38523.000324

As this response is timely filed within the one month shortened statutory period, Applicants believe that no fees are necessary in connection with the filing of this document. In the event any fees are necessary, please charge or credit any such fees, including fees for any extensions of time, to the undersigned's Deposit Account No. 50-0206.

In view of the above remarks, it is thus respectfully requested that the restriction requirement be withdrawn and that all claims be allowed to be prosecuted in the same application.

Respectfully submitted,

HUNTON & WILLIAMS

Dated: July 25, 2007

By:

Kellie L. Carden
Robert M. Schulman
Registration No. 31,196
Scott F. Yarnell
Registration No. 45,245
Kellie L. Carden
Registration No. 52,696

HUNTON & WILLIAMS LLP
Intellectual Property Department
1900 K Street, N.W. Suite 1200
Washington, DC 20006-1109
(202) 955-1500 (telephone)
(202) 778-2201 (facsimile)

SFY/KLC/ml

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference AM100904	FOR FURTHER ACTION		See item 4 below
International application No. PCT/US2004/021714	International filing date (day/month/year) 07 July 2004 (07.07.2004)	Priority date (day/month/year) 10 July 2003 (10.07.2003)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant WYETH HOLDINGS CORPORATION			

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis 1(a).																
2. This REPORT consists of a total of 7 sheets, including this cover sheet. In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) issued.																
3. This report contains indications relating to the following items: <table border="0"> <tr> <td><input checked="" type="checkbox"/> Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td><input checked="" type="checkbox"/> Box No. II</td> <td>Priority</td> </tr> <tr> <td><input type="checkbox"/> Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td><input type="checkbox"/> Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td><input checked="" type="checkbox"/> Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td><input type="checkbox"/> Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td><input type="checkbox"/> Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td><input type="checkbox"/> Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>	<input checked="" type="checkbox"/> Box No. I	Basis of the report	<input checked="" type="checkbox"/> Box No. II	Priority	<input type="checkbox"/> Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input type="checkbox"/> Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/> Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/> Box No. VI	Certain documents cited	<input type="checkbox"/> Box No. VII	Certain defects in the international application	<input type="checkbox"/> Box No. VIII	Certain observations on the international application
<input checked="" type="checkbox"/> Box No. I	Basis of the report															
<input checked="" type="checkbox"/> Box No. II	Priority															
<input type="checkbox"/> Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability															
<input type="checkbox"/> Box No. IV	Lack of unity of invention															
<input checked="" type="checkbox"/> Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement															
<input type="checkbox"/> Box No. VI	Certain documents cited															
<input type="checkbox"/> Box No. VII	Certain defects in the international application															
<input type="checkbox"/> Box No. VIII	Certain observations on the international application															
4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis.2).																

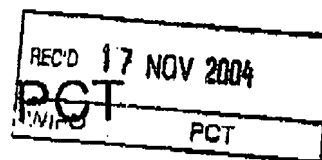
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35 Form PCT/IB/373 (January 2004)	Date of issuance of this report 16 January 2006 (16.01.2006)
	Authorized officer Athena Nickitas-Etienne Telephone No. +41 22 338 89 95

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)Applicant or agent's file reference
see form PCT/ISA/220FOR FURTHER ACTION
See paragraph 2 belowInternational application No.
PCT/US2004/021714International filing date (day/month/year)
07.07.2004Priority date (day/month/year)
10.07.2003International Patent Classification (IPC) or both national classification and IPC
C07K14.315Applicant
WYETH HOLDINGS CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA.



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 apmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Young, C

Telephone No. +49 89 2399-7877



Form PCT/ISA/237 (Cover Sheet) (January 2004)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/US2004/021714

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☒ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☒ in written format
☒ in computer readable form
 - c. time of filing/furnishing:
☒ contained in the international application as filed.
☒ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/US2004/021714**Box No. II Priority**

- 1.
- ☐
- The following document has not been furnished:

- ☐ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
- ☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-11, 23-34, 50-52, 56-65, 67-71
	No: Claims	12-22, 35-49, 53-55, 66
Inventive step (IS)	Yes: Claims	
	No: Claims	1-11, 23-34, 50-52, 56-65, 67-71
Industrial applicability (IA)	Yes: Claims	1-71
	No: Claims	

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714**Re Item V.**

The following documents are referred to in this communication:

- D1 : GUBBA SIDDESWAR ET AL: "Expression and characterization of group A Streptococcus extracellular cysteine protease recombinant mutant proteins and documentation of seroconversion during human invasive disease episodes" INFECTION AND IMMUNITY, vol. 66, no. 2, February 1998 (1998-02), pages 765-770, XP002304245 ISSN: 0019-9567
- D2 : MUSSER JAMES M ET AL: "Substitution of cysteine 192 in a highly conserved Streptococcus pyogenes extracellular cysteine protease (interleukin 1-beta convertase) alters proteolytic activity and ablates zymogen processing" INFECTION AND IMMUNITY, vol. 64, no. 6, 1996, pages 1913-1917, XP002304246 ISSN: 0019-9567
- D3: DATABASE EMBL Streptococcus pyogenes SpeB 1 June 2001 (2001-06-01), WATANABE Y: "SpeB exotoxin" XP002304250 retrieved from EBI Database accession no. Q9AJD2
- D4: KING JONATHAN ET AL: "Thermolabile folding intermediates: Inclusion body precursors and chaperonin substrates" FASEB JOURNAL, vol. 10, no. 1, 1996, pages 57-66, XP002304249 ISSN: 0892-6638
- D5: TAN S: "A MODULAR POLYCISTRONIC EXPRESSION SYSTEM FOR OVEREXPRESSING PROTEIN COMPLEXES IN ESCHERICHIA COLI" PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, US, vol. 21, 2001, pages 224-234, XP001087869 ISSN: 1046-5928

Novelty Article 33 (2) PCT

D1 discloses a method for recombinantly expressing a mature Streptococcus pyogenes exotoxin B (SpeB) polypeptide in a BL21 (DE3) E.coli host. The method of D1 is devised to express both the propolypeptide 28-145 and the mature peptide 146 through to 398, see figure 1 of D1. Moreover D1 as does D2 disclose the cysteine 192 mutation referred to in claim 4 of the present application. However, claims 1 to 11, 50-52, 56-

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714

65,67-71 are regarded as being novel over the cited art as they do not explicitly cite the use of polycistronic plasmid.

However claims 12 -22 do not refer to this feature and only make reference to conditions suitable for co-expression. In principle the method of D1 or D2 would be suitable for co-expression thus this authority consider that these disclosure anticipate novelty for these claims.

Claims 23-34 relate to any recombinant expression system for SpeB however in particular defining steps to overcome solubility problems. These are not stated expressis verbis in D1 or D2 and for this reason these claims are viewed as being novel.

This is in stark contrast to the product claims 35-44 which essentially relate to SpeB polypeptide per se or as an immunogenic composition. This authority consider that the protein of D1 or D3 anticipates these claims.

The methods of immunization of claims 45-49 are anticipated by the rabbit vaccination of D1 as described on page 767.

Claims 53-55,66 are not novel over recombinant plasmids and hosts of D1.

Inventive step Article 33 (2) PCT.

The closest prior art is identified as being D1. D1 discloses a recombinant expression method for SpeB both propolypeptide and mature protein. Essentially the present invention differs from D1 in that it discloses the use of a polycistronic vector system. Moreover claims 23-34 furthermore introduce steps to avoid protein insolubility.

The objective problem is thus defined as being

"the provision of an alternative method for recombinant SpeB expression"

The solution being the use of a polycistronic vector system and for claims 23-34 steps the use of chaperones to overcome solubility problems. In each case the skilled person would be inclined to combine the teachings in the art with the methods of D1 or D2 without the use of inventive skill.

For example to overcome solubility problems as defined in claims 23-34 the skilled person would simply combine the recombinant expression methods for Spe of D1 and D2 with D4.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714

Additionally, D5 describes the use of polycistronic vectors systems for co-expression and in combination with D1 or D2 would enable the skilled person to derive the present alleged invention without the use of inventive skill.

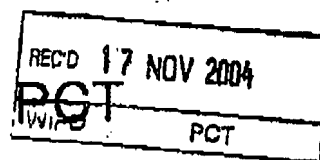
In short claims 1-11, 23-34, 50-52, 56-65, 67-71 recognized above as being novel do not possess inventive merit and as such do not meet the requirements of Article 33 (3) PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)Applicant's or agent's file reference
see form PCT/ISA/220FOR FURTHER ACTION
See paragraph 2 belowInternational application No.
PCT/US2004/021714International filing date (day/month/year)
07.07.2004Priority date (day/month/year)
10.07.2003International Patent Classification (IPC) or both national classification and IPC
C07K14.315Applicant
WYETH HOLDINGS CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 86.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80288 Munich
Tel. +49 89 2369 - 0 Tx: 523665 epmu d
Fax +49 89 2399 - 4465

Authorized Officer

Young, C

Telephone No. +49 89 2399-7877



Form PCT/ISA/237 (Cover Sheet) (January 2004)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/US2004/021714**Box No. I Basis of the opinion**

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed.
 - ☒ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**International application No.
PCT/US2004/021714**Box No. II Priority**

1. ☐ The following document has not been furnished:
- ☐ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).
- Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.
4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Yes: Claims	1-11, 23-34, 50-52, 56-65, 67-71
	No: Claims	12-22, 35-49, 53-55, 66
Inventive step (IS)	Yes: Claims	
	No: Claims	1-11, 23-34, 50-52, 56-65, 67-71
Industrial applicability (IA)	Yes: Claims	1-71
	No: Claims	

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714**Re Item V.**

The following documents are referred to in this communication:

- D1 : GUBBA SIDDESWAR ET AL: "Expression and characterization of group A Streptococcus extracellular cysteine protease recombinant mutant proteins and documentation of seroconversion during human invasive disease episodes" INFECTION AND IMMUNITY, vol. 66, no. 2, February 1998 (1998-02), pages 765-770, XP002304245 ISSN: 0019-9567
- D2 : MUSSER JAMES M ET AL: "Substitution of cysteine 192 in a highly conserved Streptococcus pyogenes extracellular cysteine protease (interleukin 1-beta convertase) alters proteolytic activity and ablates zymogen processing" INFECTION AND IMMUNITY, vol. 64, no. 6, 1996, pages 1913-1917, XP002304246 ISSN: 0019-9567
- D3: DATABASE EMBL Streptococcus pyogenes SpeB 1 June 2001 (2001-06-01), WATANABE Y: "SpeB exotoxin" XP002304250 retrieved from EBI Database accession no. Q9AJD2
- D4: KING JONATHAN ET AL: "Thermolabile folding intermediates: Inclusion body precursors and chaperonin substrates" FASEB JOURNAL, vol. 10, no. 1, 1996, pages 57-66, XP002304249 ISSN: 0892-6638
- D5: TAN S: "A MODULAR POLYCISTRONIC EXPRESSION SYSTEM FOR OVEREXPRESSING PROTEIN COMPLEXES IN ESCHERICHIA COLI" PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, US, vol. 21, 2001, pages 224-234, XP001087869 ISSN: 1046-5928

Novelty Article 33 (2) PCT

D1 discloses a method for recombinantly expressing a mature Streptococcus pyogenes exotoxin B (SpeB) polypeptide in a BL21(DE3) E.coli host. The method of D1 is devised to express both the propolypeptide 28-145 and the mature peptide 146 through to 398, see figure 1 of D1. Moreover D1 as does D2 disclose the cysteine 192 mutation referred to in claim 4 of the present application. However, claims 1 to 11, 50-52, 56-

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714

65,67-71 are regarded as being novel over the cited art as they do not explicitly cite the use of polycistronic plasmid.

However claims 12 -22 do not refer to this feature and only make reference to conditions suitable for co-expression. In principle the method of D1 or D2 would be suitable for co-expression thus this authority consider that these disclosure anticipate novelty for these claims.

Claims 23-34 relate to any recombinant expression system for SpeB however in particular defining steps to overcome solubility problems. These are not stated expressis verbis in D1 or D2 and for this reason these claims are viewed as being novel.

This is in stark contrast to the product claims 35-44 which essentially relate to SpeB polypeptide per se or as an immunogenic composition. This authority consider that the protein of D1 or D3 anticipates these claims.

The methods of immunization of claims 45-49 are anticipated by the rabbit vaccination of D1 as described on page 767.

Claims 53-55,66 are not novel over recombinant plasmids and hosts of D1.

Inventive step Article 33 (2) PCT.

The closest prior art is identified as being D1. D1 discloses a recombinant expression method for SpeB both polypeptide and mature protein. Essentially the present invention differs from D1 in that it discloses the use of a polycistronic vector system. Moreover claims 23-34 furthermore introduce steps to avoid protein insolubility.

The objective problem is thus defined as being

"the provision of an alternative method for recombinant SpeB expression"

The solution being the use of a polycistronic vector system and for claims 23-34 steps the use of chaperones to overcome solubility problems. In each case the skilled person would be inclined to combine the teachings in the art with the methods of D1 or D2 without the use of inventive skill.

For example to overcome solubility problems as defined in claims 23-34 the skilled person would simply combine the recombinant expression methods for Spe of D1 and D2 with D4.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2004/021714

Additionally, D5 describes the use of polycistronic vectors systems for co-expression and in combination with D1 or D2 would enable the skilled person to derive the present alleged invention without the use of inventive skill.

In short claims 1-11, 23-34, 50-52, 56-65, 67-71 recognized above as being novel do not possess inventive merit and as such do not meet the requirements of Article 33 (3) PCT.